Investigation of the Toxic & Teratogenic Effects of GRAS Substances to the Developing Chicken Embryo-Report of the in-house investigations of **Calcium Chloride** in the developing chicken embryo 4/24/74

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MEMORANDUM

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION

TO : Mr. Alan Spiher
GRAS Review Branch, HFF-335

DATE: April 24, 1974

THRU : Dr. Leo Friedman, Director
Division of Toxicology, HFF-150

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M Jacqueline Verrett

SUBJECT: Investigation of the Toxic and Teratogenic Effects of GRAS Substances to the Developing Chicken Embryo.

Attached is the report of the in-house investigations of Calcium Chloride in the developing chicken embryo.

Investigations of the Toxic and Teratogenic Effects of GRAS Substances to the Developing Chicken Embryo: Calcium Chloride

Protocol:

Calcium Chloride (1) was tested for toxic and teratogenic effects to the developing chicken embryo under four sets of conditions. It was administered in water as the solvent by two routes and at two stages of embryonic development; via the air cell at pre-incubation (0 hours) and at 96 hours of incubation, and via the yolk at 0 hours and 96 hours using techniques that have been described previously (2,3).

Groups of fifteen or more eggs were treated under these four conditions at several dose levels until a total of seventy-five to one hundred eggs per level was reached for all levels allowing some hatch. Groups of comparable size were treated with the solvent at corresponding volumes and untreated controls were also included in each experiment.

After treatment, all eggs were candled daily and non-viable embryos removed. Surviving embryos were allowed to hatch. Hatched chicks and non-viable embryos were examined grossly for abnormalities (internally and externally) as well as for toxic responses such as edema and hemorrhage. All abnormalities were tabulated.

Results:

The results obtained are presented in tables 1 through 4 for each of the four conditions of test.

Column 1 and 2 give the dose administered in milligrams per egg and milligrams per kilogram, respectively. (The milligrams per kilogram figure is based on an average egg weight of fifty grams.)

Column 3 is the total number of eggs treated.

Column 4 is the percent mortality, i.e., total non-viable divided by total treated eggs.

Column 5 is the total number of abnormal birds expressed as a percentage of the total eggs treated. This includes all abnormalities observed and also toxic responses such as edema, hemorrhage, hypopigmentation of the down and other disorders such as feather abnormalities, significant growth retardation, cachexia or other nerve discorders.

Column 6 is the total number of birds having a structural abnormality of the head, viscera, limbs, or body skeleton expressed as percentage of the total eggs treated. Toxic responses and disorders such as those noted for column 5 are not included.

Column 3 through 6 have been corrected for accidental deaths if any occurred. Included in these columns are comparable data for the solvent-treated eggs and the untreated controls.

The mortality data in column 4 have been examined for a linear relationship between the probit percent mortality versus the logarithm of the dose according to the procedures of Finney (4). The results obtained are indicated at the bottom of each table.

The data of columns 4, 5 and 6 have been analyzed using the Chi Square test for significant differences from the solvent background. Each dose level is compared to the solvent value and levels that show differences at the 5% level or lower are indicated by an asterisk in the table.

Discussion:

Calcium Chloride showed slight embryotoxicity when levels above 25.0 mg/kg were injected for all four modes of treatment. The LD-50 for air cell treatment at 0 hours is estimated at 195.20 mg/kg (9.76 mg/egg) while air cell treatment at 96 hours resulted in an estimate of 36.61 mg/kg (1.831 mg/egg). Treatment via the yolk at both 0 and 96 hours showed a regression of mortality with dose but the slopes of the lines were not significantly ($p=0.05$) different from zero.

There were serious anomalies scattered through the dose levels of all four modes of treatment, but the incidences of such anomalies involving the head, limbs, viscera, or skeleton were low except in one instance: 100 mg/kg, air cell at 0 hours. Some of the serious anomalies seen included coloboma, dysgnathia, microphthalmia, buphthalmia, microblepharia, cleft palate, anaphthalmia, exencephaly, and celosomia. The untreated birds had one serious abnormality, dysgnathia. Solvent-treated controls had two serious anomalies: one bird with microphthalmia for air cell at 96 hours, and one bird with hypoplasia of the head for yolk treatment at 0 hours.

It is concluded from these data that under these conditions of test calcium is neither particularly embryotoxic nor teratogenic to the developing chicken embryo.

1. Calcium Chloride, Lot #44090, J.T. Baker Chemical Co.

2. McLaughlin, J., Jr., Marliac, J.-P., Verrett, M. Jacqueline, Mutchler, Mary K., and Fitzhugh, O.G., (1963) Toxicol. Appl. Pharmacol. 5, 760-770.

3. Verrett, M.J., Marliac, J.-P., and McLaughlin, J., Jr., (1964) JAOAC 47, 1002 - 1006.

4. Finney, D.J., (1964) Probit Analysis, 2nd Ed., Cambridge Press, Cambridge, Appendix I.

Table 1

Calcium Chloride

Air Cell at 0 Hours

Dose		Number of Eggs	**Percent Mortality	Percent Abnormal	
ml/egg	mg/kg			Total	Structural
10.00	200.00	105	59.04*	8.57*	3.80
5.00	100.00	105	50.47*	17.14*	9.52*
2.50	50.00	104	36.53*	6.73*	0.96
0.50	10.00	105	15.23	5.71*	1.90
0.1250	2.50	104	25.00*	3.84	0.96
Water		169	14.20	0.59	0.59
Controls		323	20.43	1.23	1.23

**LD_{50} 195.20 mg/kg (9.76 mg/egg)

* Significantly different from solvent ($p \leq 0.05$)

Table 2

Calcium Chloride

Air Cell at 96 Hours

Dose		Number of Eggs	** Percent Mortality	Percent Abnormal	
Mg/kg	mg/kg			Total	Structural
5.00	100.00	100	99.00*	0.00	0.00
2.50	50.00	100	84.00*	0.00	0.00
1.250	25.00	100	29.00	7.00*	3.00
0.250	5.00	100	15.00	4.00	4.00
0.06250	1.25	100	20.00	3.00	1.00
Water		115	22.60	0.86	0.86
Controls		323	20.43	1.23	1.23

**LD_{50} 36.6165 mg/kg (1.831 mg/egg)

* Significantly different from solvent ($p \leq 0.05$)

Table 3

Calcium Chloride

Yolk at 0 Hours

Dose		Number of Eggs	** Percent Mortality	Percent Abnormal	
[illegible]	mg/kg			Total	Structural
10.00	200.00	100	80.00*	7.00	6.00
5.00	100.00	128	81.25*	2.34	1.56
2.50	50.00	129	68.99*	1.55	0.77
1.250	25.00	30	36.66	10.00	13.33
0.500	10.00	100	78.00*	5.00	2.00
0.250	5.00	30	46.66	6.66	0.00
0.1250	2.50	100	76.00*	1.00	1.00
0.06250	1.25	30	40.00	6.66	3.33
Water		125	31.20	2.40	2.40
Controls		323	20.43	1.23	1.23

**Slope is not significantly different from zero ($p=0.05$)

* Significantly different from solvent ($p \leq 0.05$)

Table 4

Calcium Chloride

Yolk at 96 Hours

Dose µl/egg	mg/kg	Number of Eggs	**Percent Mortality	Percent Abnormal Total	Percent Abnormal Structural
5.00	100.00	70	45.71*	5.71	2.85
2.50	50.00	70	44.28*	4.28	0.00
1.250	25.00	70	44.28*	8.57	4.28
0.250	5.00	70	40.00	7.14	4.28
0.06250	1.25	70	32.85	1.42	1.42
Water		75	25.33	1.33	0.00
Controls		323	20.43	1.23	1.23

**Slope is not significantly different from zero (p=0.05)

* Significantly different from solvent ($p \leq 0.05$)